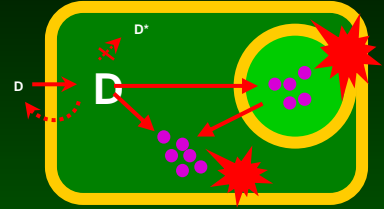




# Models of Intracellular Antibiotic Transport



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[www.facm.ucl.ac.be](http://www.facm.ucl.ac.be)



# Why intracellular antibiotics ?

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antibiotic

The Cell

bacteria

Black Box...

# Intracellular antibiotics: the issues

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1. which bacteria and where ?



2. which antibiotics accumulate ?

3. influx vs efflux ?

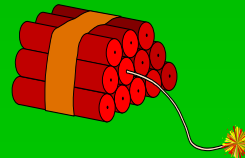
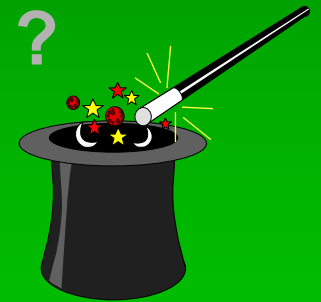
3. where are antibiotics in cells ?

4. intracellular expression of activity ?

5. bacterial responsiveness ?

6. cooperation with host defenses ?

7. any toxicity ?



# Which antibiotics accumulate in cells ?

---

- **beta-lactams:  $\leq 1x$**
- **aminoglycosides:  $<1$  to  $2x$**
- **ansamycins:  $2-3x$**
- **tetracyclines:  $2-4x$**
- **fluoroquinolones:  $5 - 20x$**
- **macrolides:  $4$  to  $> 100x$  \***
- **glycopeptides:  $1$  to  $400x$  !! \*\***

\* azithromycin, ketolides

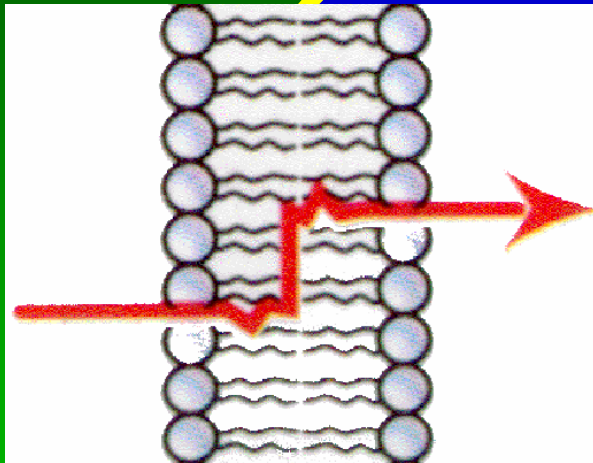
\*\* oritavancin

# How do antibiotics penetrate in cells ?

---

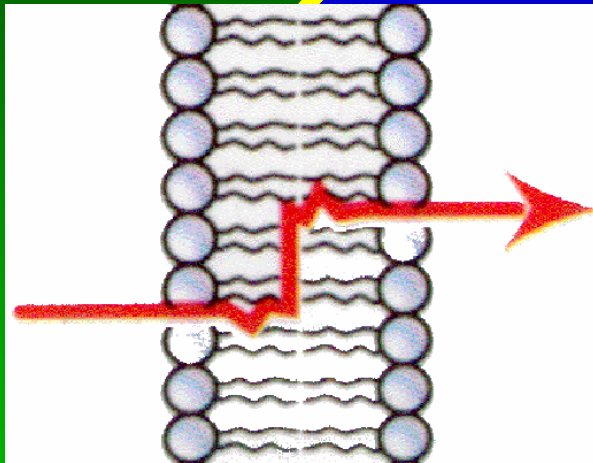
- **trans-membrane influx**
  - ▶ **diffusion**
  - ▶ **carrier mediated**
- **endocytosis**

# Entry by diffusion ...



- **amphiphilic compounds**
- **fast**
- **non-saturable**
- **no competition by analogues**

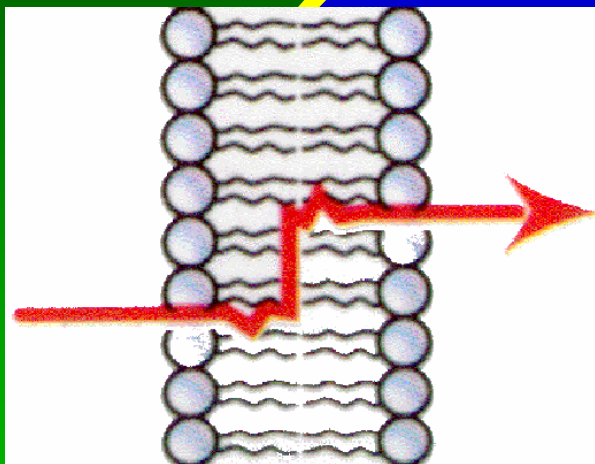
# Entry by diffusion: some examples...



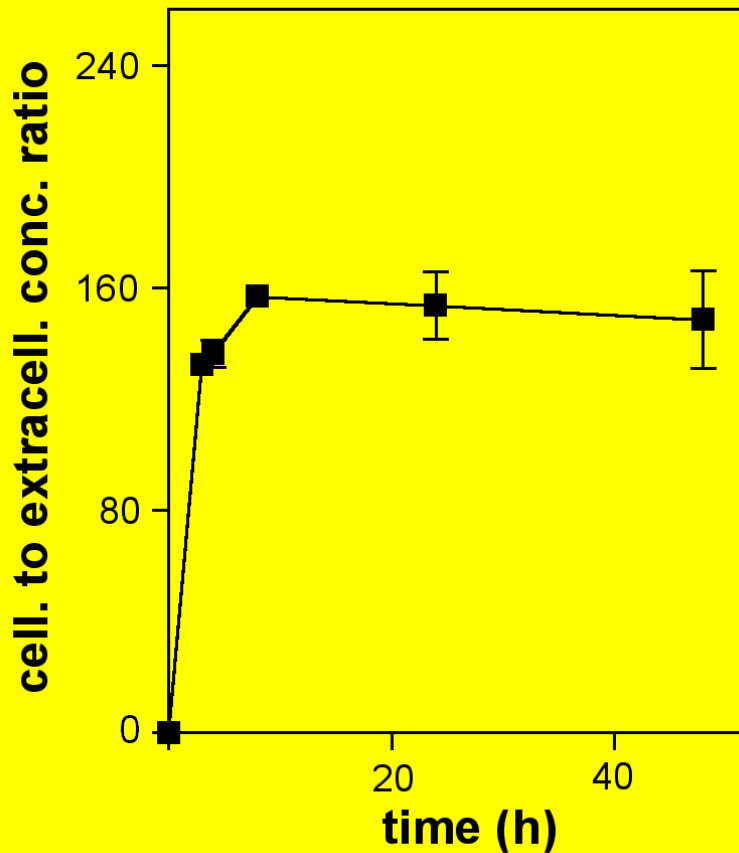
- ▶ macrolides
- ▶ fluoroquinolones
- ▶ tetracyclines
- ▶ ansamycines
- ▶  $\beta$ -lactams,
- ▶ ...

# Entry of azithromycin...

diffusion



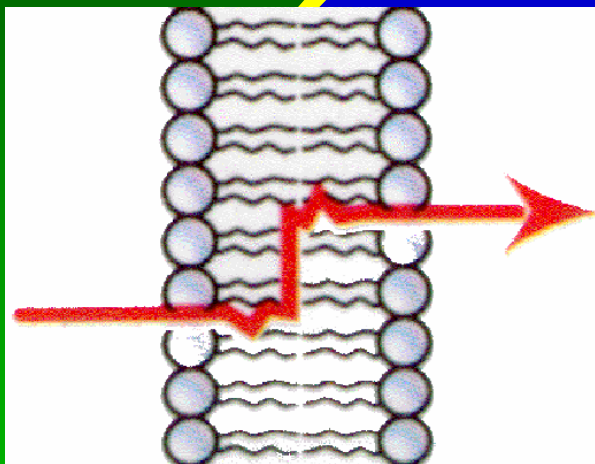
### Azithromycin accumulation in rat embryo fibroblats



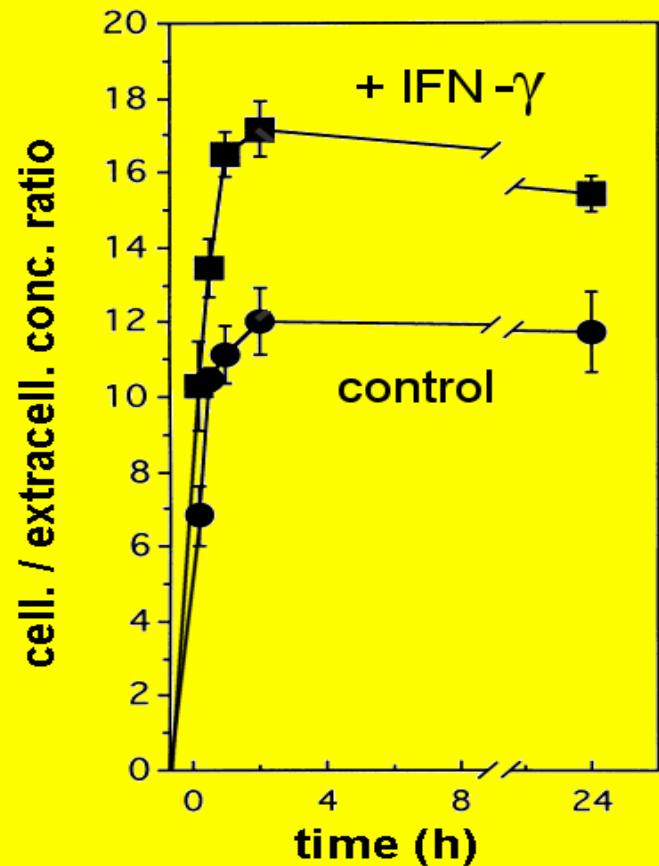
Tyteca et al., EJCB, 2001, in press

# Entry of sparfloxacin ...

diffusion



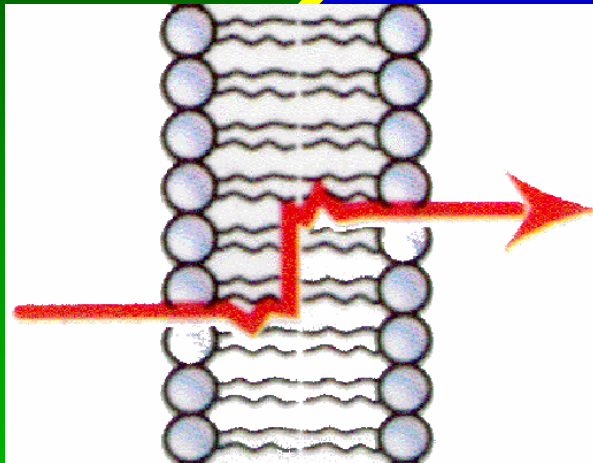
## Sparfloxacin accumulation in THP-1 macrophages



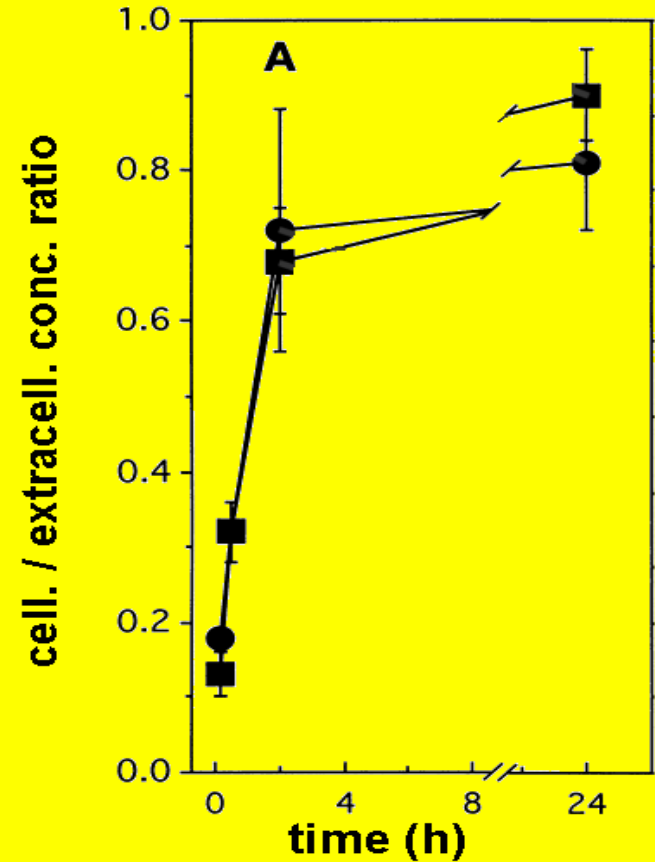
Quadrhiri et al., AAC, 1999

# Entry of ampicillin ...

diffusion

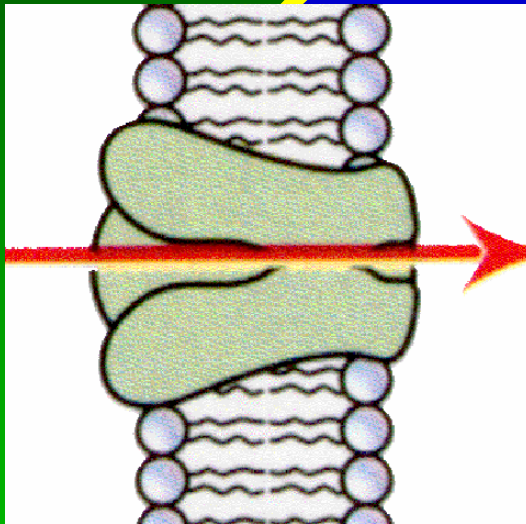


### Ampicillin accumulation in THP-1 macrophages



Quadrhiri et al., AAC, 1999

# Carrier-mediated influx ?



- specific structure
- (energy-dependent)
- saturable
- competition by analogues

**only limited evidence for specific transporters is available so far**

# Carrier-mediated influx ?

## the case of HSR-903

0022-3565/99/2891-0079\$03.00/0

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JPET 289:79-84, 1999

Vol. 289, No. 1

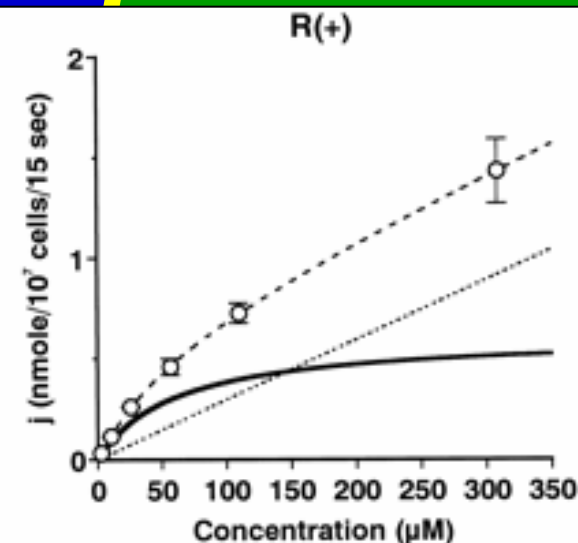
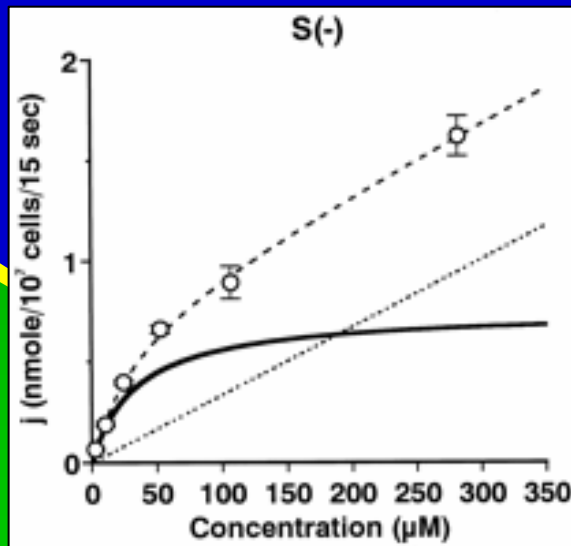
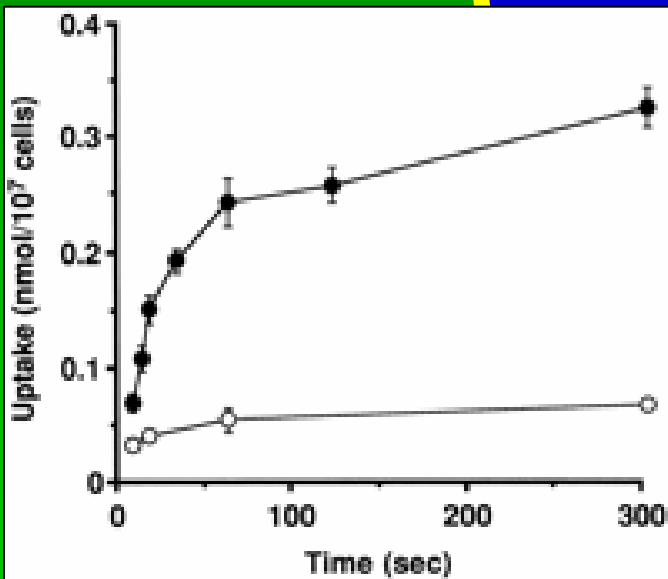
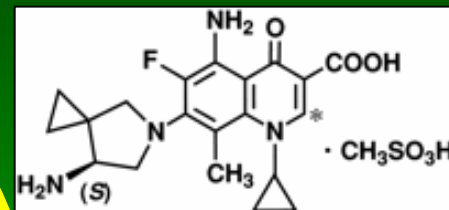
Printed in U.S.A.

### Carrier-Mediated Lung Distribution of HSR-903, a New Quinolone Antibacterial Agent<sup>1</sup>

MITSUO MURATA, IKUMI TAMAI, YOSHIMICHI SAI, OSAMU NAGATA, HIDEO KATO, and AKIRA TSUJI

Department of Pharmacobio-Dynamics, Faculty of Pharmaceutical Sciences, Kanazawa University (M.M., I.T., Y.S., A.T.), Kanazawa, Japan; Research and Development Division, Hokuriku Seiyaku Co. (M.M., O.N., H.K.), Inokuchi, Fukui, Japan; and Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Kawaguchi, Saitama, Japan (I.T., Y.S., A.T.)

Accepted for publication October 27, 1998 This paper is available online at <http://www.jpnet.org>



# Carrier-mediated influx ?

## the case of HSR-903

TABLE 1

Inhibitory effect on [<sup>14</sup>C]HSR-903 (10 μM) uptake by unlabeled HSR-903 (*S*-isomer) and its *R*-isomer

	Inhibitor concentration	Uptake <sup>a</sup>
	μM	pmol/10 <sup>7</sup> cells
Control	0	20.26 ± 1.44
HSR-903 ( <i>S</i> -isomer)	50	15.23 ± 0.72 <sup>b</sup>
	500	10.49 ± 0.79 <sup>c</sup>
<i>R</i> -isomer	50	17.68 ± 0.85
	500	11.45 ± 1.31 <sup>c</sup>

Each value indicates mean ± S.E. from four experiments.

<sup>a</sup> Uptake was determined at 15 s.

<sup>b</sup> *P* < .05.

<sup>c</sup> *P* < .01, by ANOVA.

TABLE 3

Inhibition of [<sup>14</sup>C]HSR-903 (10 μM) uptake by various quinolones (500 μM)

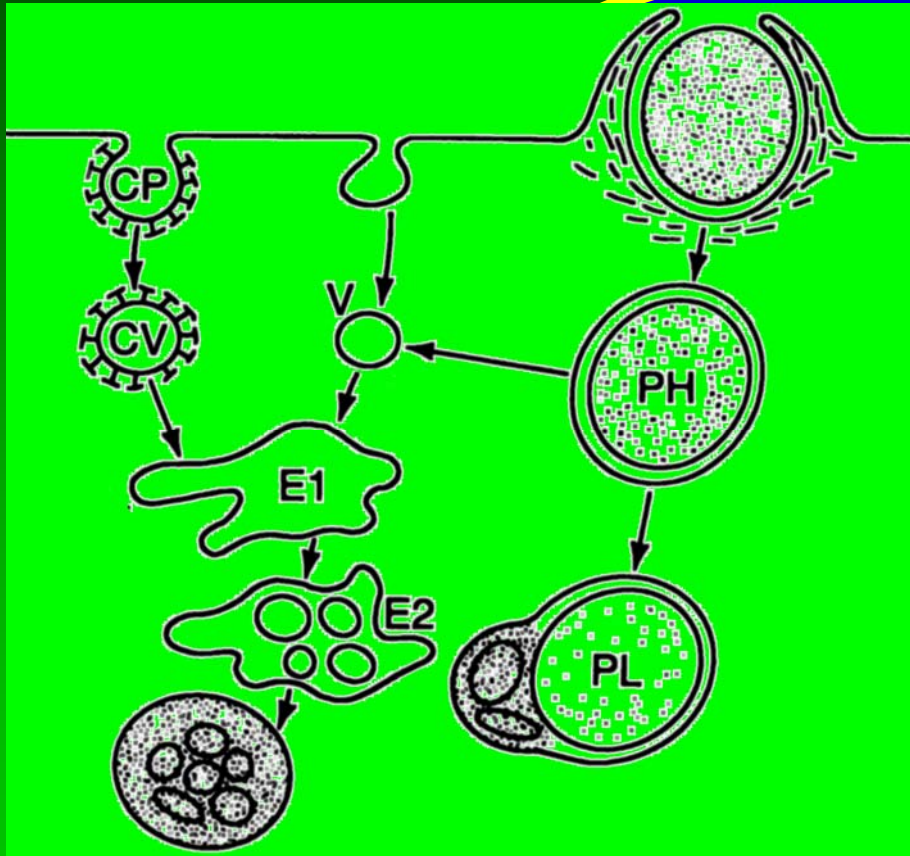
Inhibitor	Uptake
	% of control
HSR-903	51.71 ± 3.78 <sup>a</sup>
Grepafloxacin	58.91 ± 4.21 <sup>a</sup>
Sparfloxacin	77.01 ± 3.73 <sup>b</sup>
Levofloxacin	79.53 ± 5.31
Fleroxacin	89.85 ± 9.42
Tosufloxacin	117.97 ± 4.34
Lomefloxacin	99.23 ± 6.03
Ofloxacin	88.35 ± 9.81
Enoxacin	84.91 ± 6.17
Norfloxacin	90.81 ± 5.14

Each value indicates mean ± S.E. from four experiments

<sup>a</sup> *P* < .01.

<sup>b</sup> *P* < .05, by ANOVA.

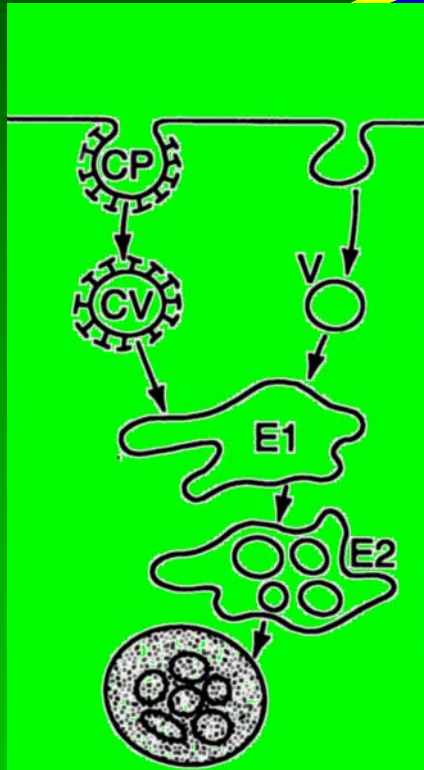
# Endocytosis...



- non-permeant drugs
- slow unless membrane-bound, or receptor-mediated
- confined to vacuolar system

# Endocytosis: examples...

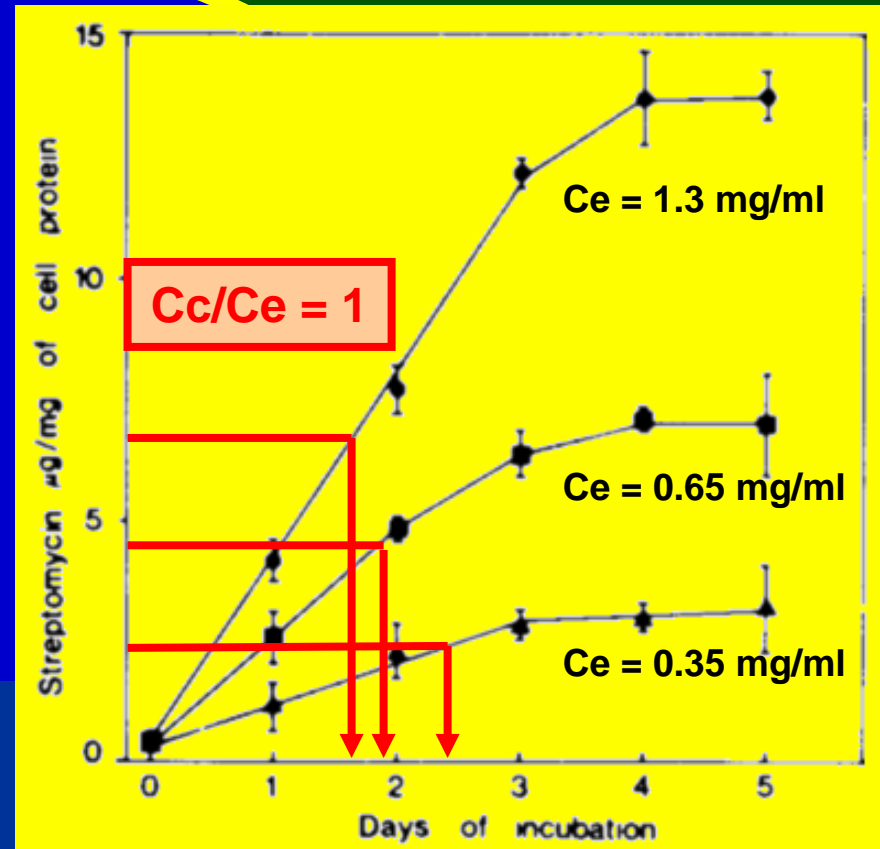
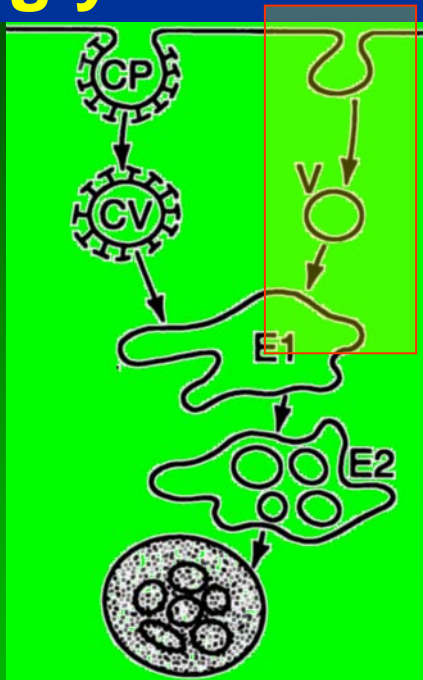
## pinocytosis



- ▶ aminoglycosides
- ▶ glycopeptides (?)

# Fluid-phase endocytosis...

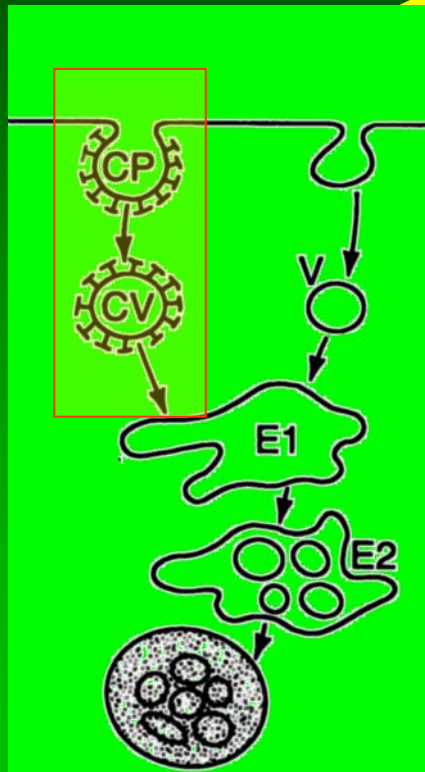
## aminoglycosides in fibroblasts or macrophages



Tulkens & Trouet, 1978

- ▶ Slow (days...)
- ▶ ill-effective (2-4 fold)
- ▶ non-saturable

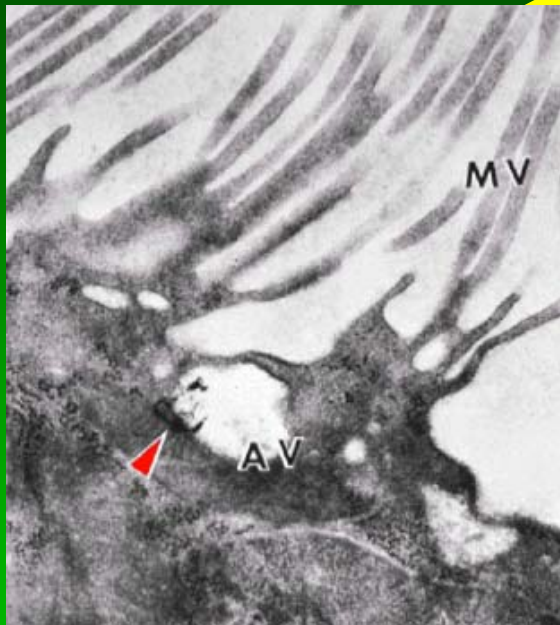
# Receptor-mediated endocytosis...



- ▶ fast (days...)
- ▶ very effective (100 –fold or more)
- ▶ saturable ...

# Receptor-mediated endocytosis...

## entry of aminoglycosides in kidney tubular cells



### binding to

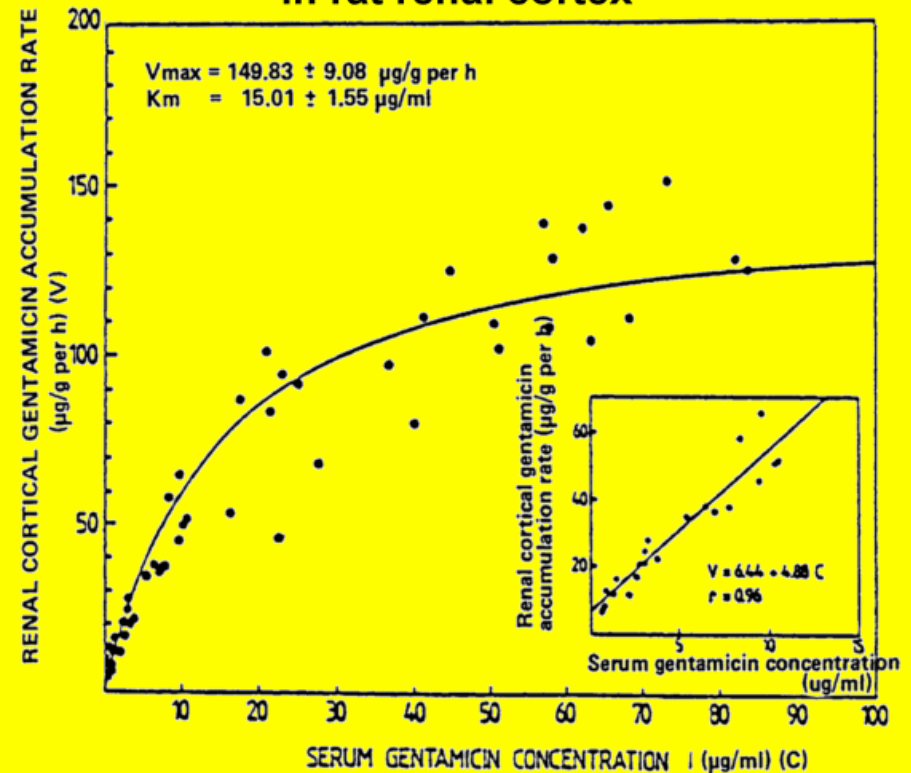
- **megalyn**

(Moeströp et al., 1995)

- **acidic phospholipids**

(Humes et al, 1983)

### In vivo accumulation of gentamicin in rat renal cortex

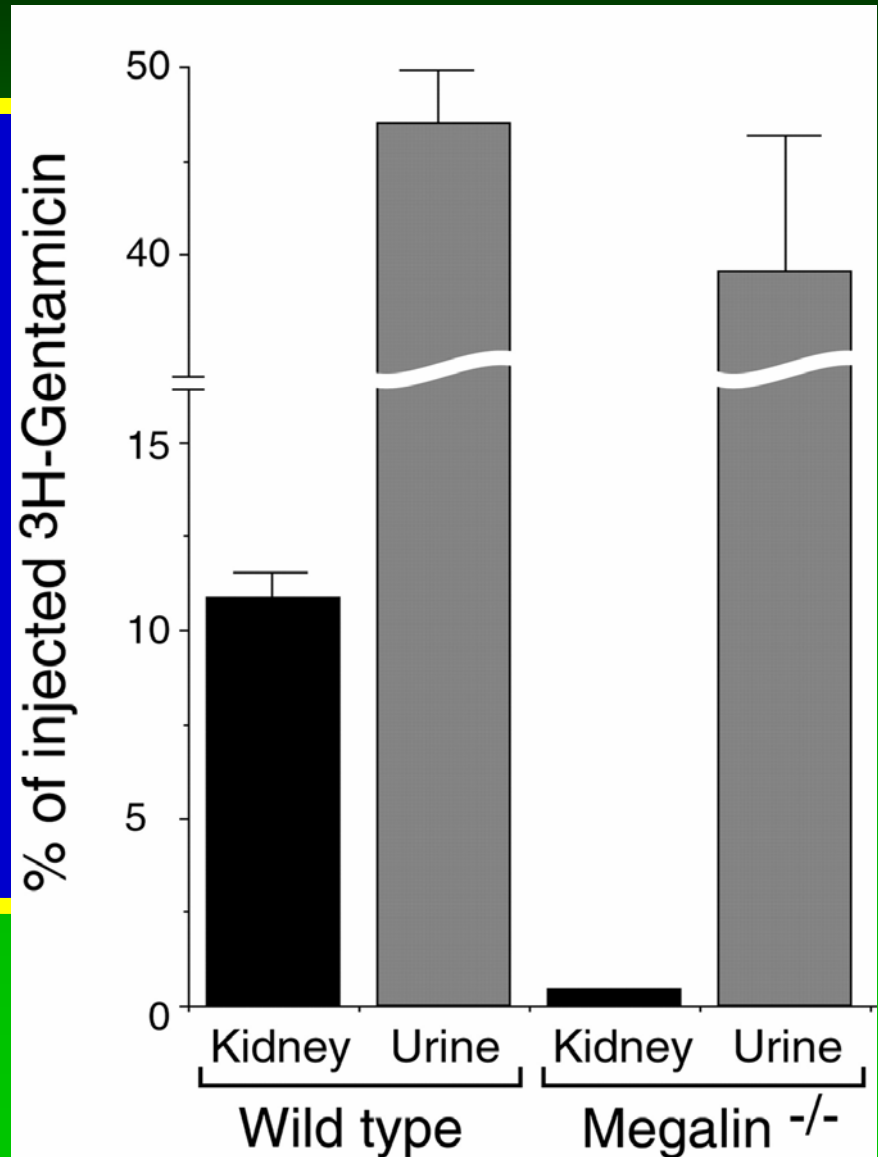


Giuliano *et al.*, J. Pharm. Exp. Ther., 1986

# Receptor-mediated endocytosis...

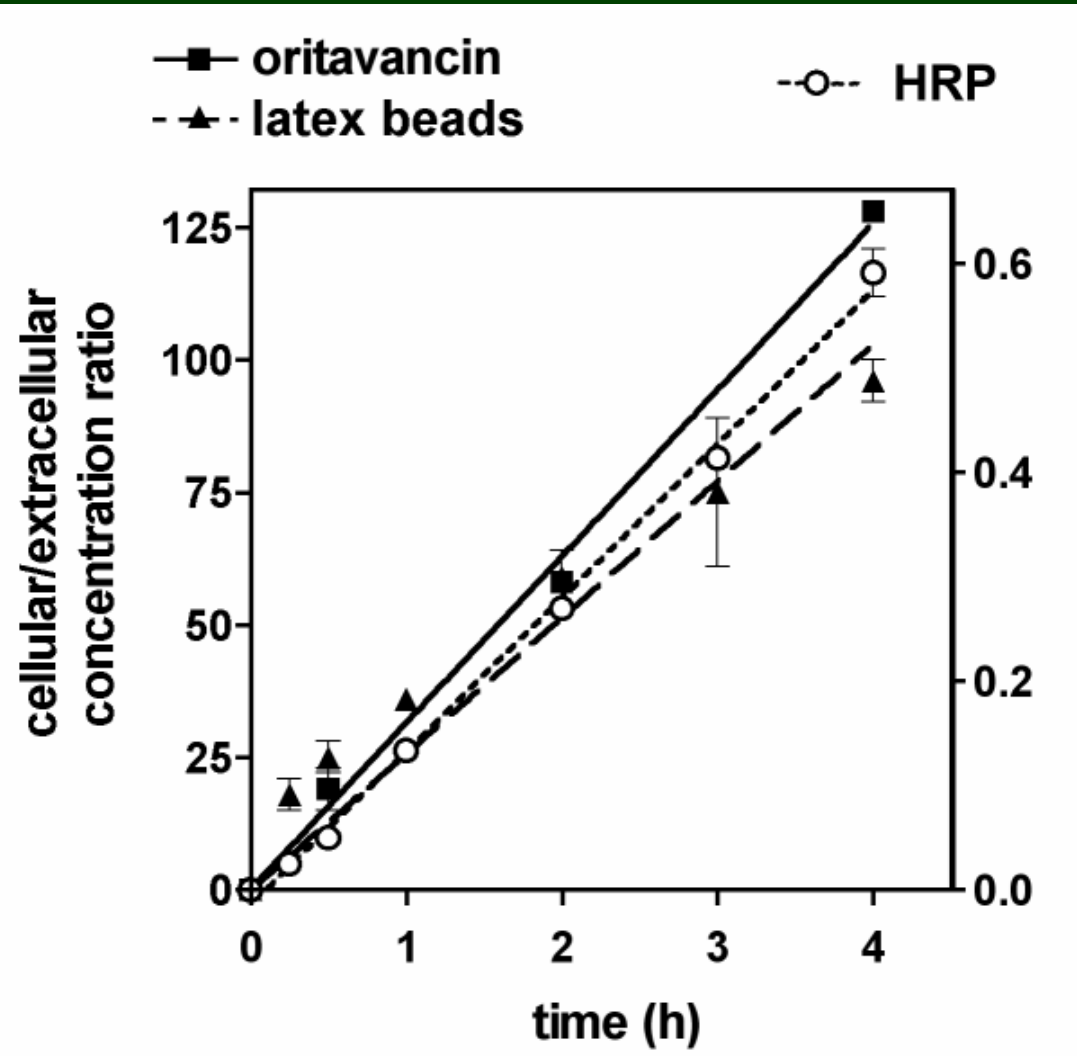
Mice deficient in megalin do not accumulate gentamicin in kidney

Schmitz et al., J. Biol. Chem. 277:618-622, 2002



# Membrane-binding-mediated endocytosis ? ...

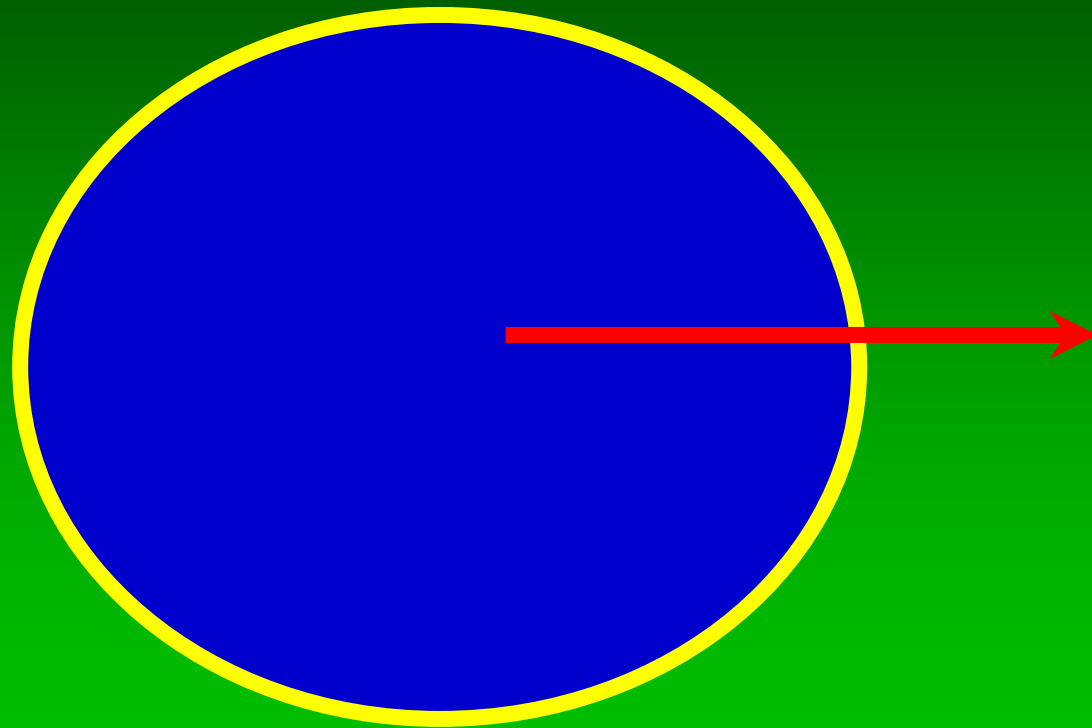
- ▶ very effective if tight binding (100 –fold or more)
- ▶ continuous over time ...



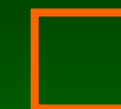
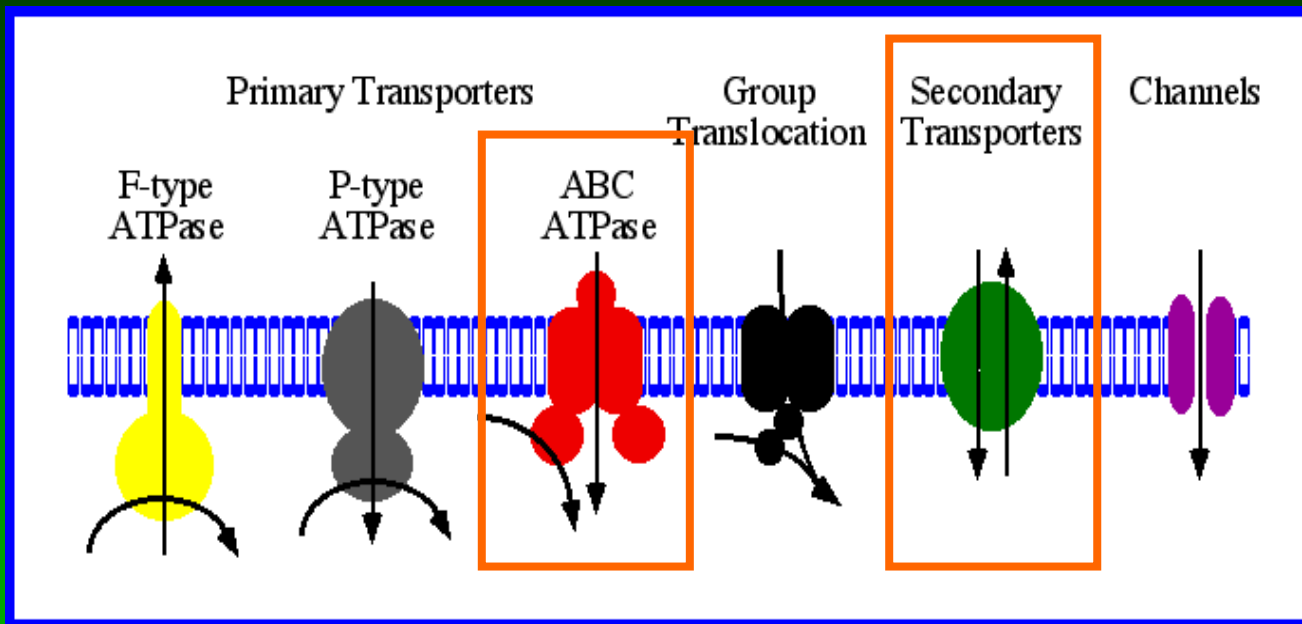
Van Bambeke et al., AAC (2004) 48:2853-2860

# Antibiotics efflux ?

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# Transporters - data bases



main  
drug  
transporters

Transporter Page Links

- Transport Classification
- Genomic Transport Analysis
- Transport Family Phylogeny
- Biotoools
- Grasp-DNA
- Align
- Software

<http://www-biology.ucsd.edu/~msaier/transport/>

Saier, 2000



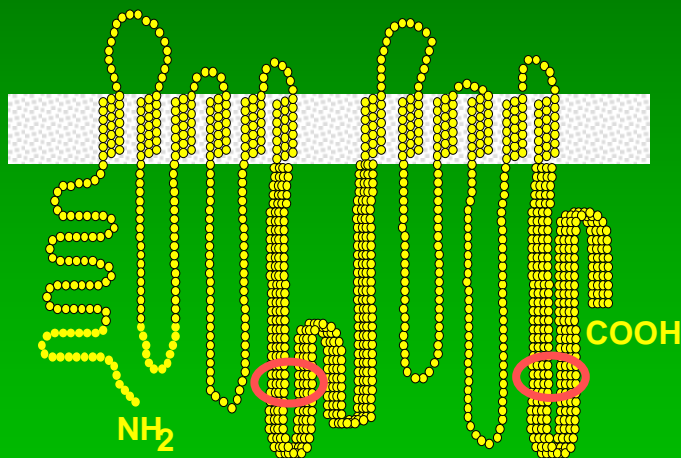
# Transporters involved in the efflux of antibiotics from eucaryotic cells

<b>superfamily</b>	<b>transporter</b>	<b>physiol. substrates</b>	<b>antibiotics</b>
<b>ABC</b>	<b>MDR1</b>	<b>phospholipids</b>	<b>fluoroquinolones macrolides <math>\beta</math>-lactams tetracyclines streptogramins</b>
	<b>MRP1</b>	<b>phospholipids leukotrienes conjugates</b>	<b>fluoroquinolones macrolides rifamycins</b>
	<b>MRP2</b>	<b>conjugates</b>	<b>fluoroquinolones <math>\beta</math>-lactams</b>
<b>MFS</b>	<b>NPT1</b>	<b>phosphates</b>	<b><math>\beta</math>-lactams</b>
<b>OAT</b>	<b>OATP1</b>	<b>bile salts steroids</b>	<b><math>\beta</math>-lactams</b>

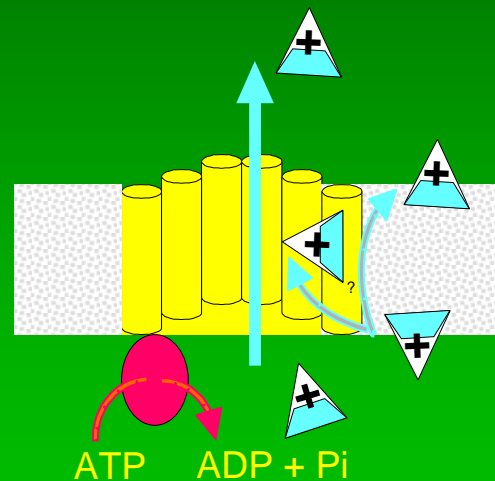
# Most frequent antibiotic-pumps in eucaryotes (1/2)

## Multiple Drug Resistance (MDR also known as PgP)






### TOPOLOGY



### MECHANISM



### ANTIBIOTICS

-  tetracyclines
-  fluoroquinolones
-  erythromycin
-  lincosamides
-  rifampicin
  
-  chloramphenicol
  
-  aminoglycosides

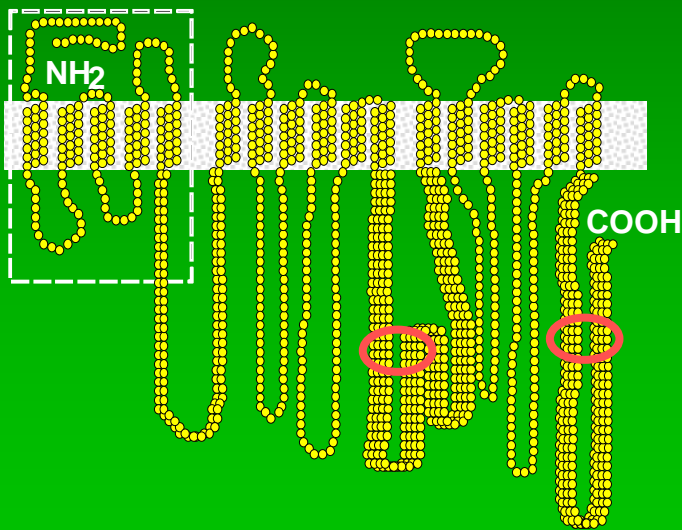
Van Bambeke et al., Biochem. Pharmacol. 2000

# Most frequent antibiotic-pumps in eucaryotes (2/2)

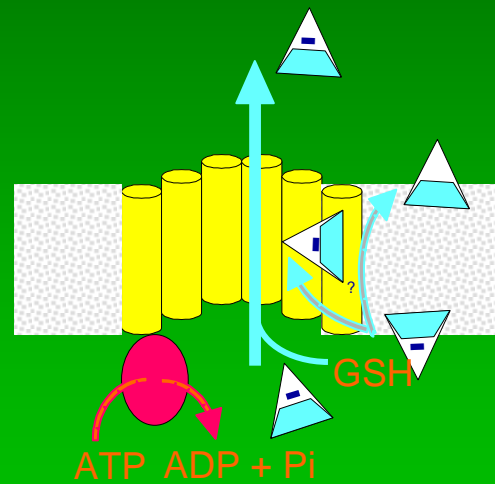


## Multidrug Resistance Proteins (MRP)

### TOPOLOGY



### MECHANISM



### ANTIBIOTICS



fluoroquinolones

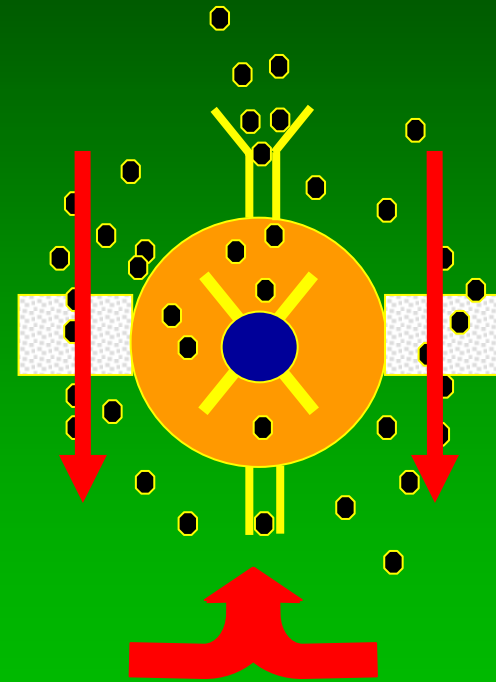
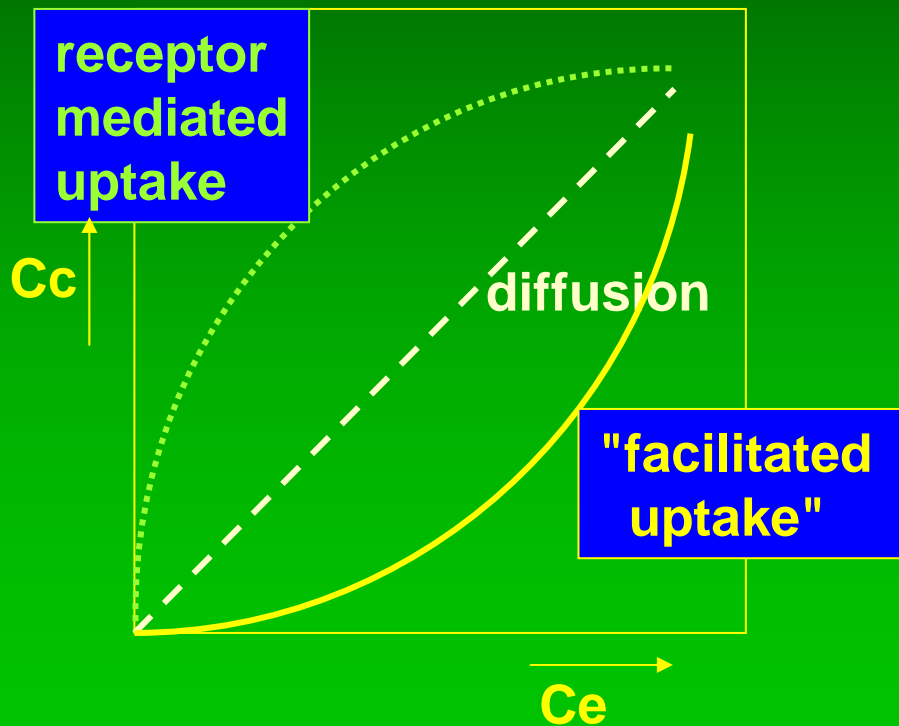


tetracyclines  
macrolides

Van Bambeke et al., Biochem. Pharmacol. 2000

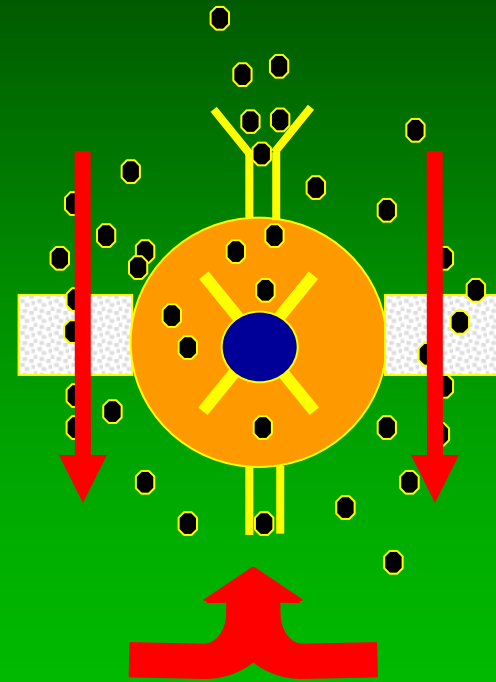
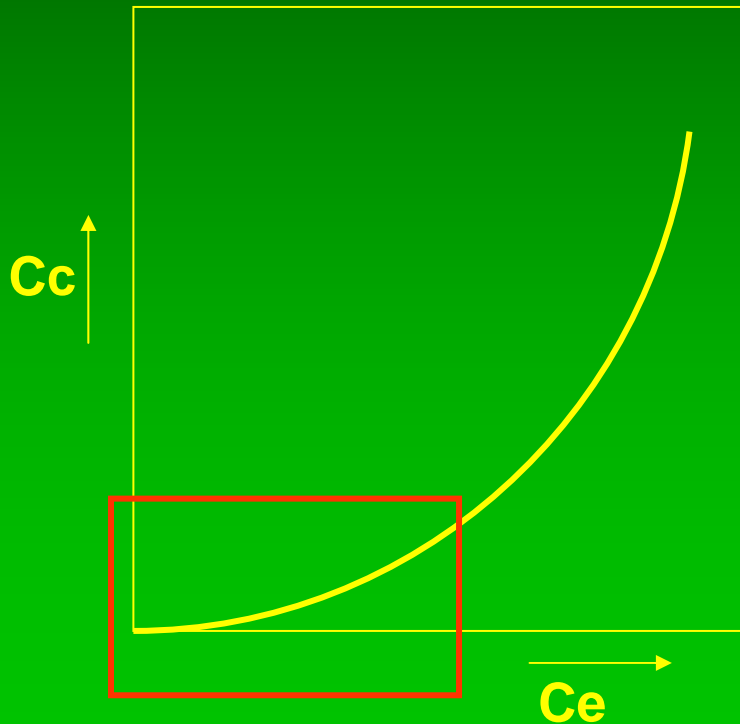
# Evidencing active efflux ...

non linear accumulation kinetics ...



# Evidencing active efflux ...

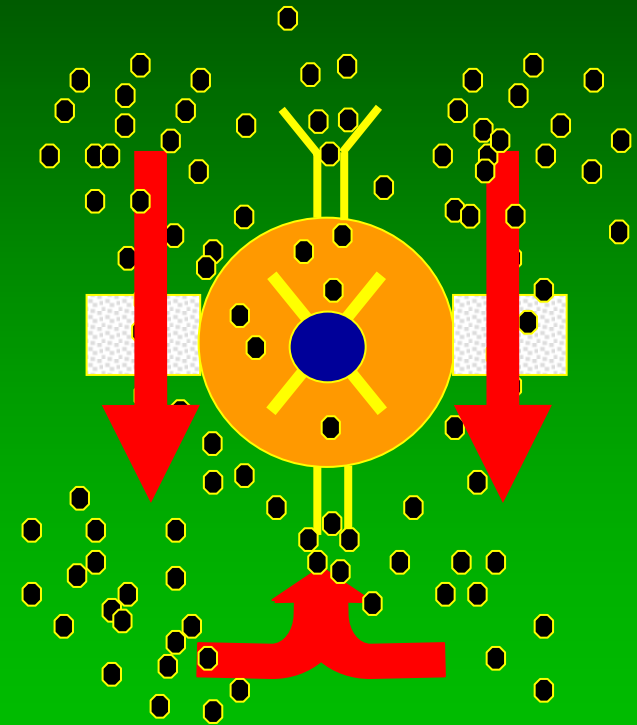
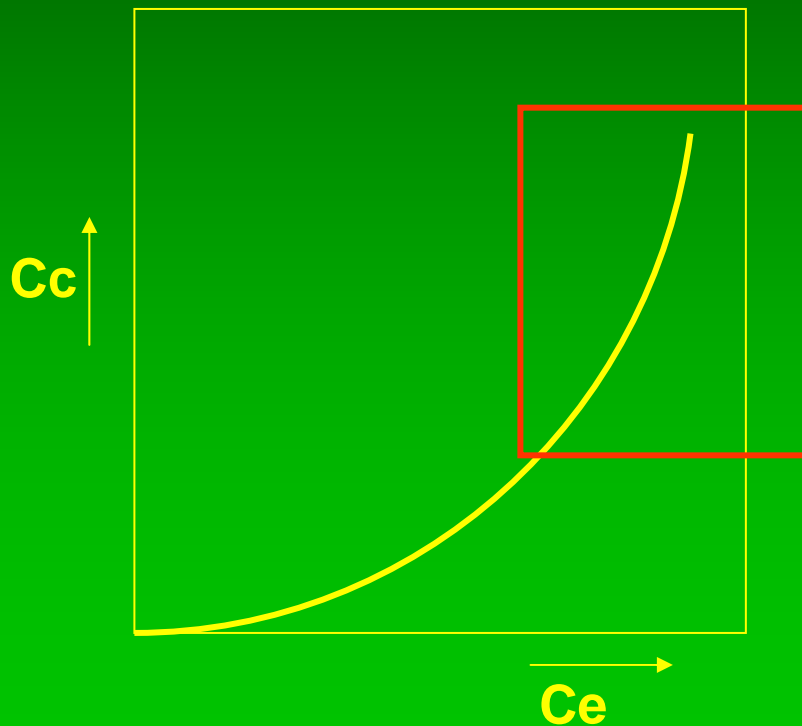
non linear accumulation kinetics ...



at low concentrations, most of the drug is reexported ...

# Evidencing active efflux ...

non linear accumulation kinetics ...

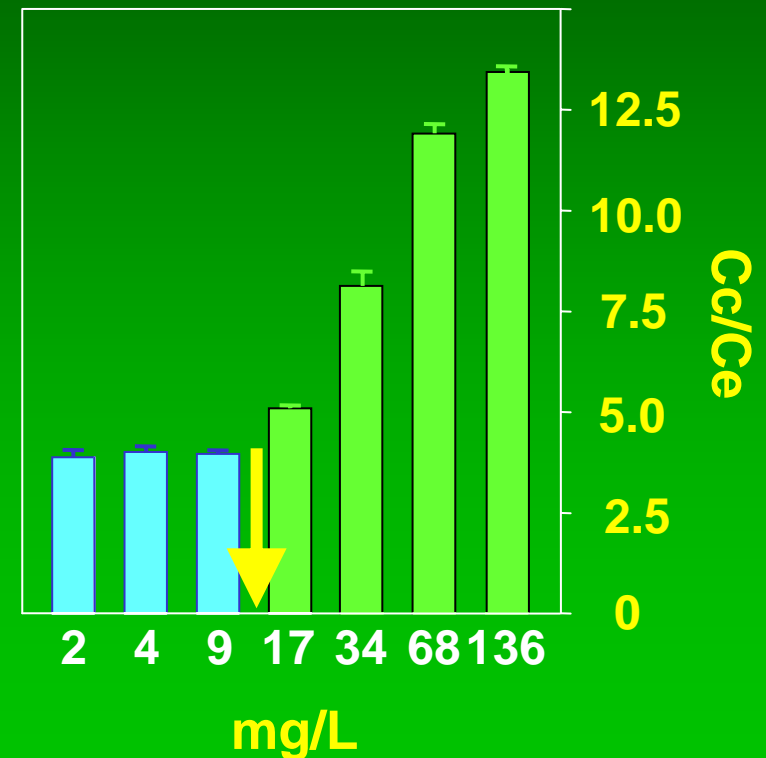
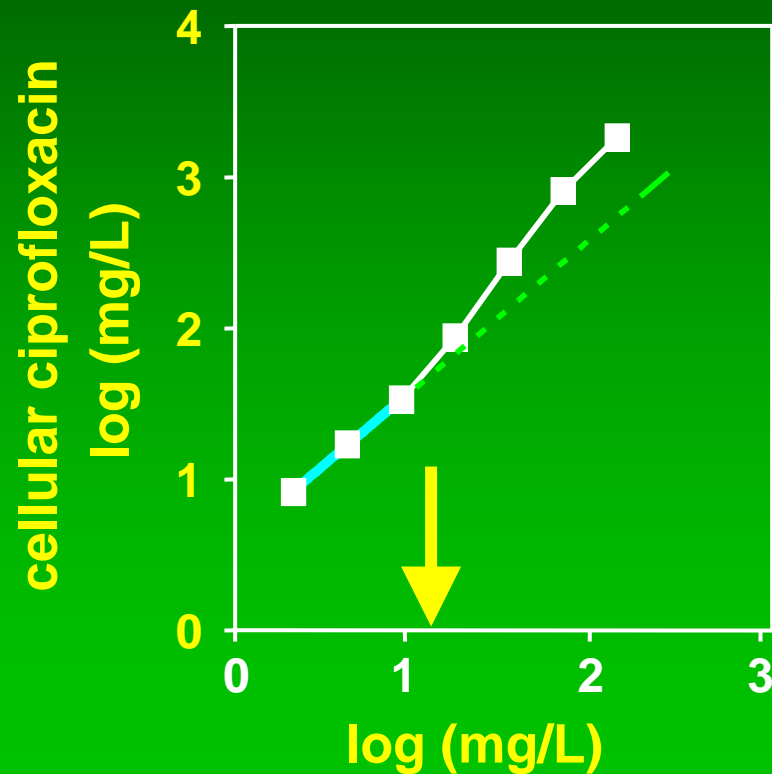


at large concentrations, efflux becomes saturated

# Evidencing efflux of ciprofloxacin

Ciprofloxacin accumulation in J774 macrophages is facilitated upon increase of its extracellular concentration

Michot et al., AAC (2004) 48:2673-2682



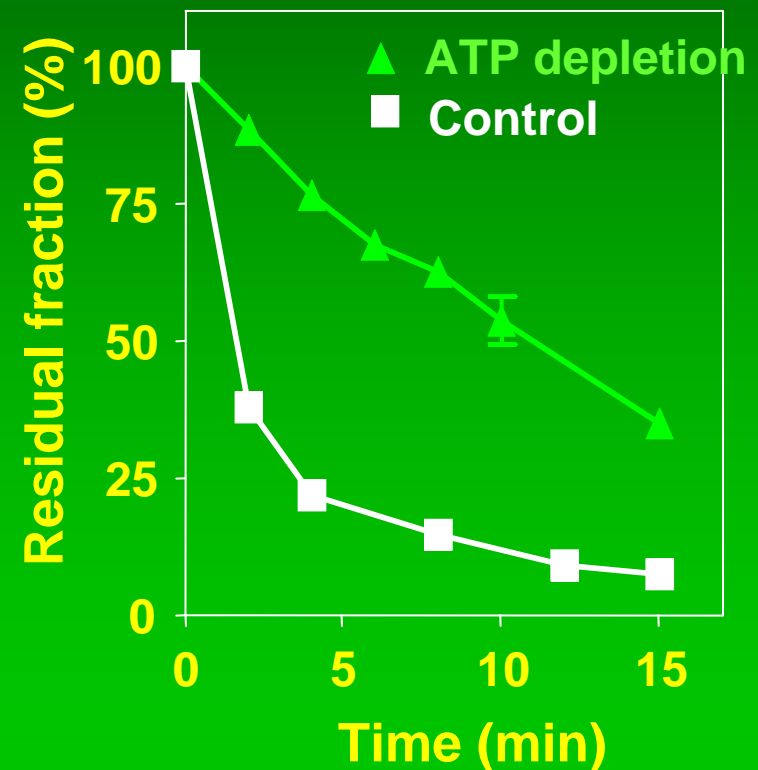
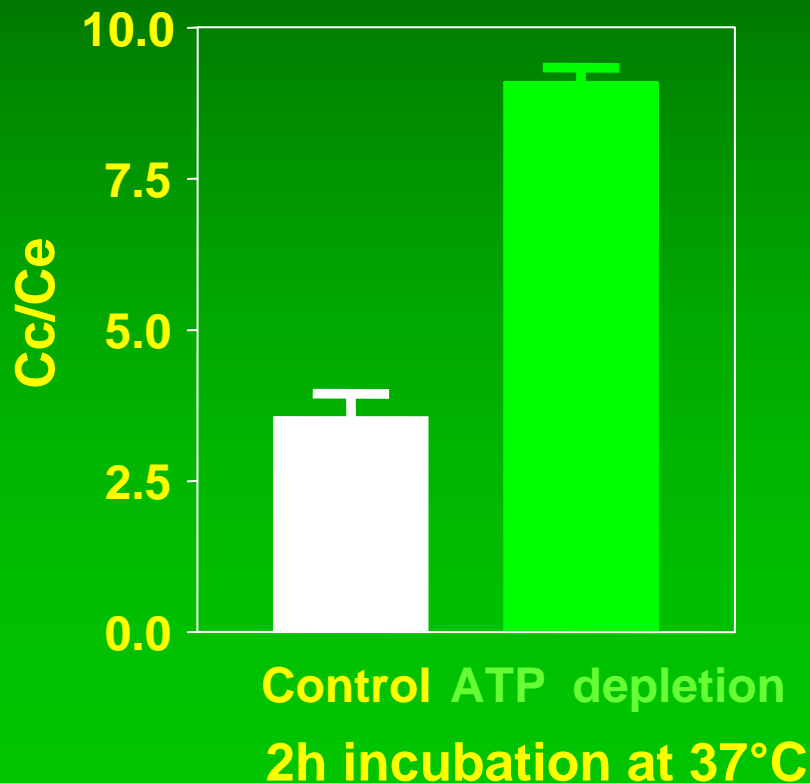
extracellular [ciprofloxacin] - 2h incubation at 37°C

# Characterization of the transporter(s)

ATP depletion increases ciprofloxacin accumulation and decreases ciprofloxacin efflux in J774 macrophages

Michot et al., AAC (2004) 48:2673-2682

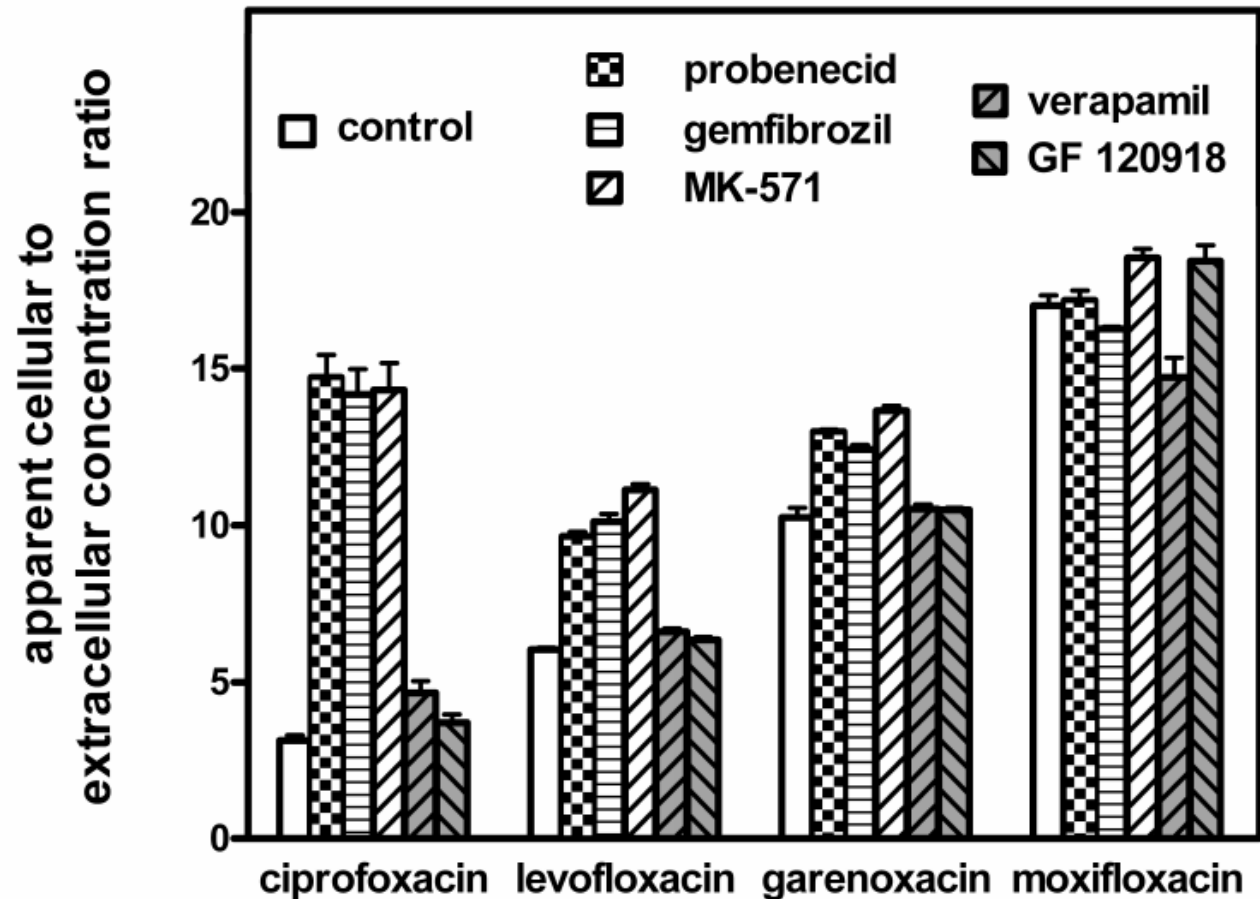
$C_e = 50 \mu\text{M}$  (17  $\mu\text{g/ml}$ )



# Efflux vary among closely related derivatives and may be impaired by (apparently) unrelated substances

## accumulation of quinolones in J774 macrophages and influence of P-gp and MRP inhibitors

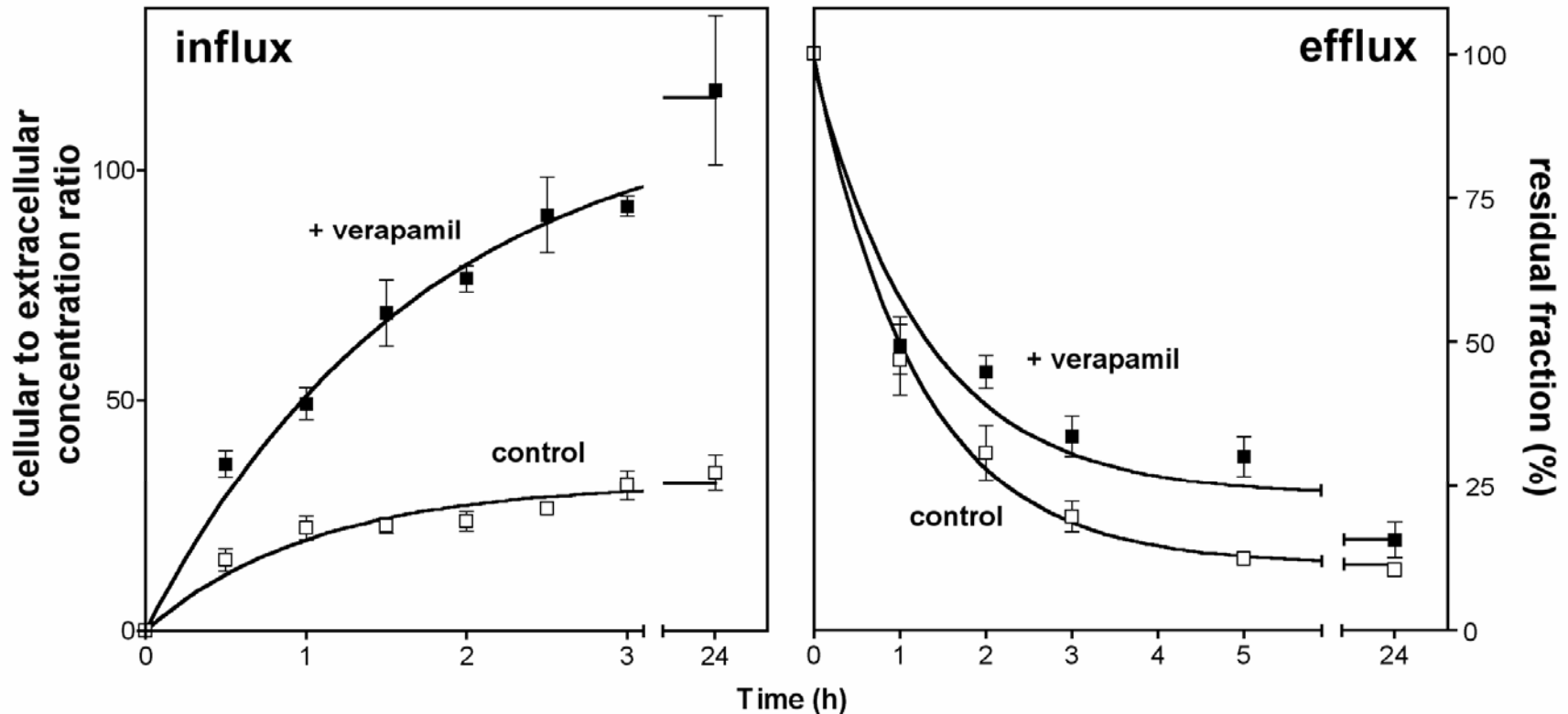
Michot et al. AAC  
(2005) 49:2429-2437



# Evidencing efflux of azithromycin macrolides (through P-gp) ...



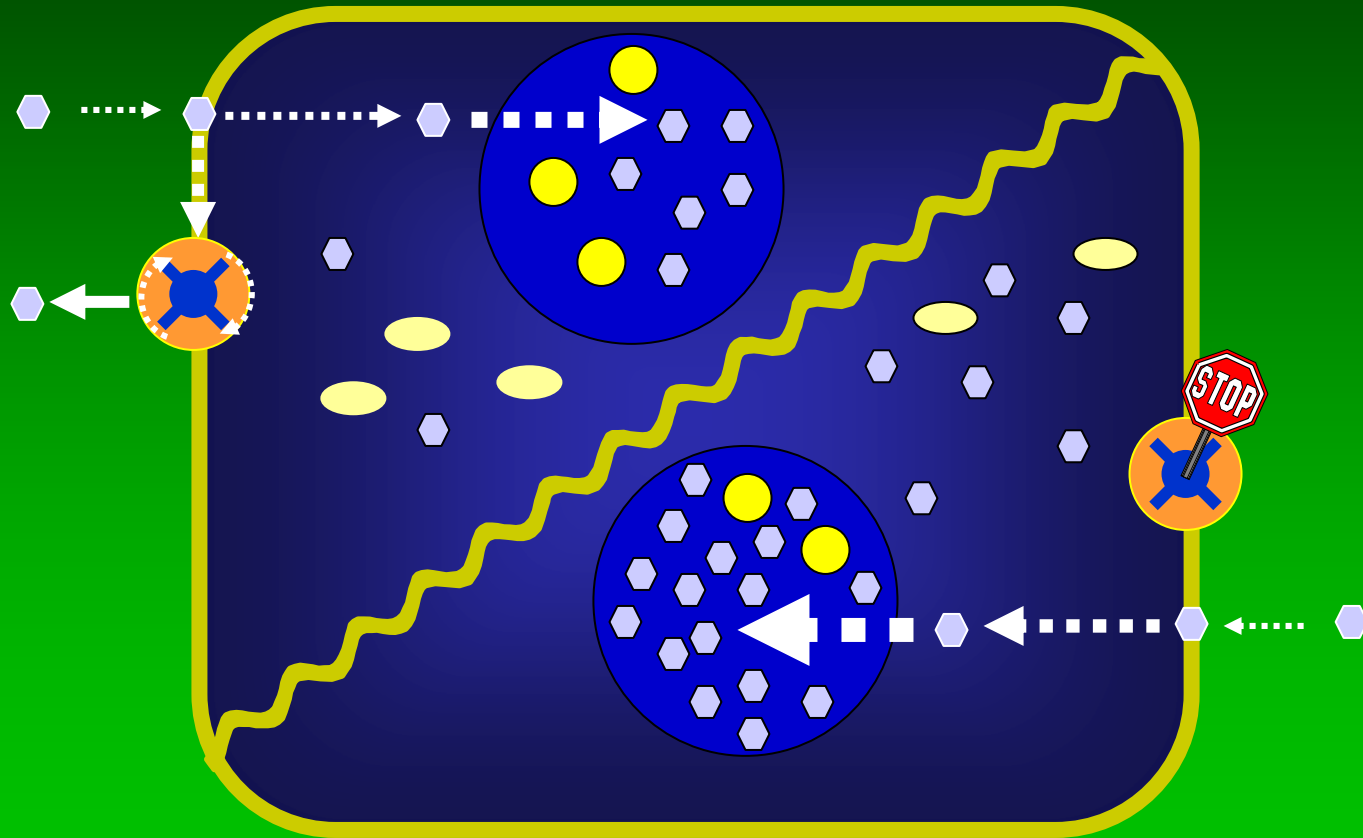
Kinetics of influx and efflux of azithromycin in J774 murine macrophages with or without 20  $\mu$ M verapamil.



Seral et al., AAC (2003) 47:1047-1051

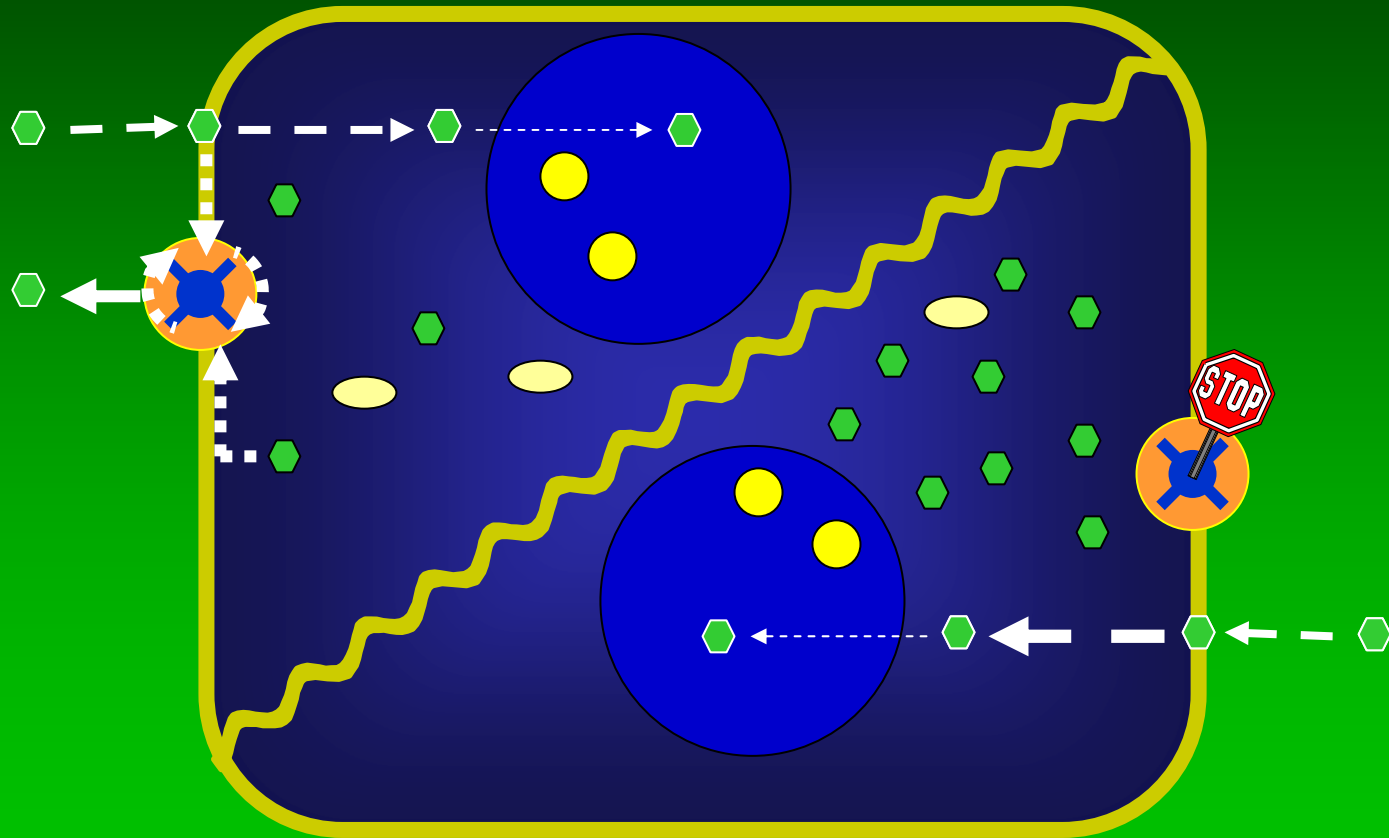
# Azithromycin follows the a 'kick-back' model

Gaj *et al.* (1998) *Biochem. Pharmacol.* 55:1199-211

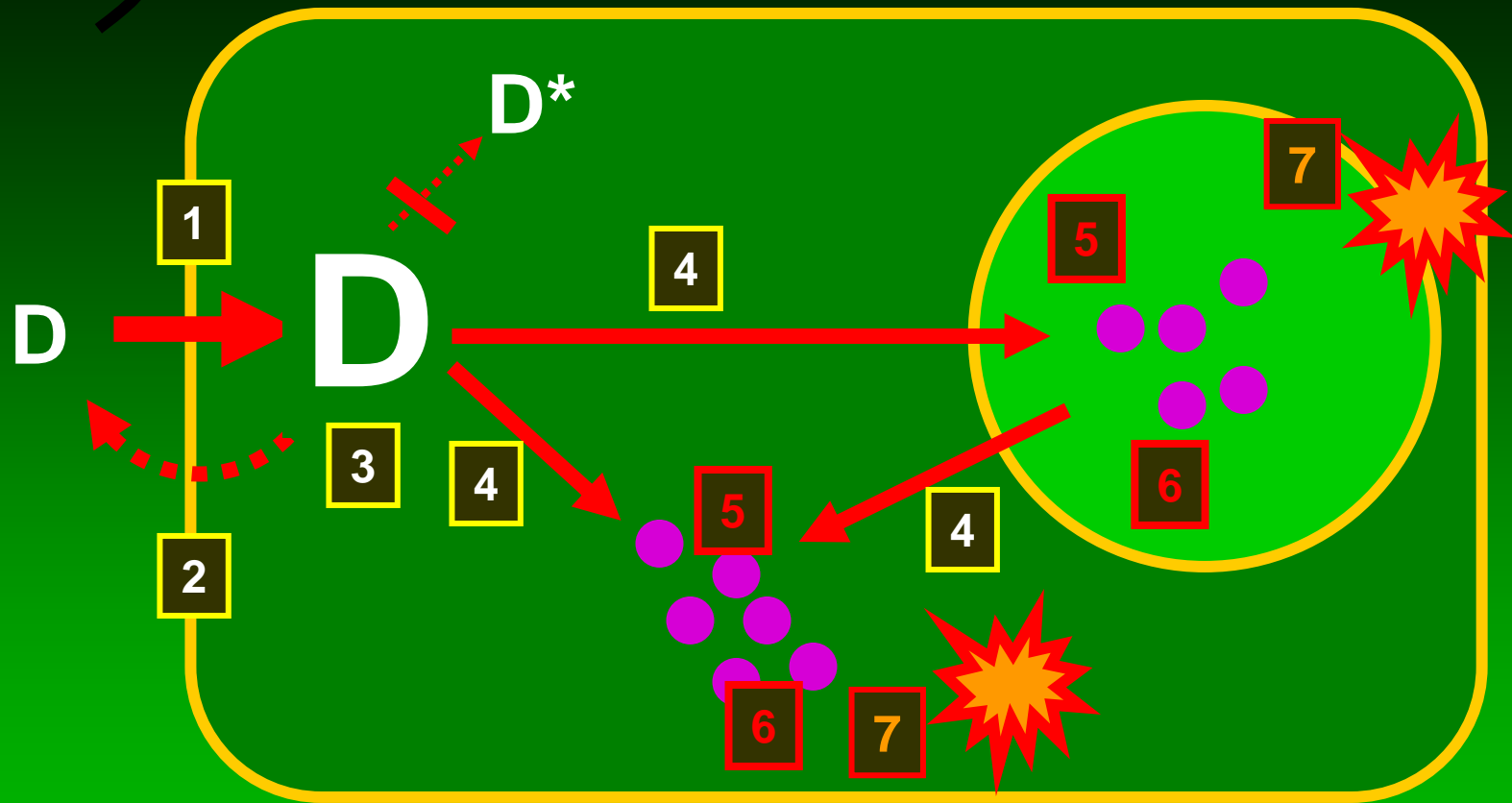


# Ciprofloxacin is following the classical model

Kolaczkowski & Goffeau (1997) Pharmacol. Ther. 76:219-42



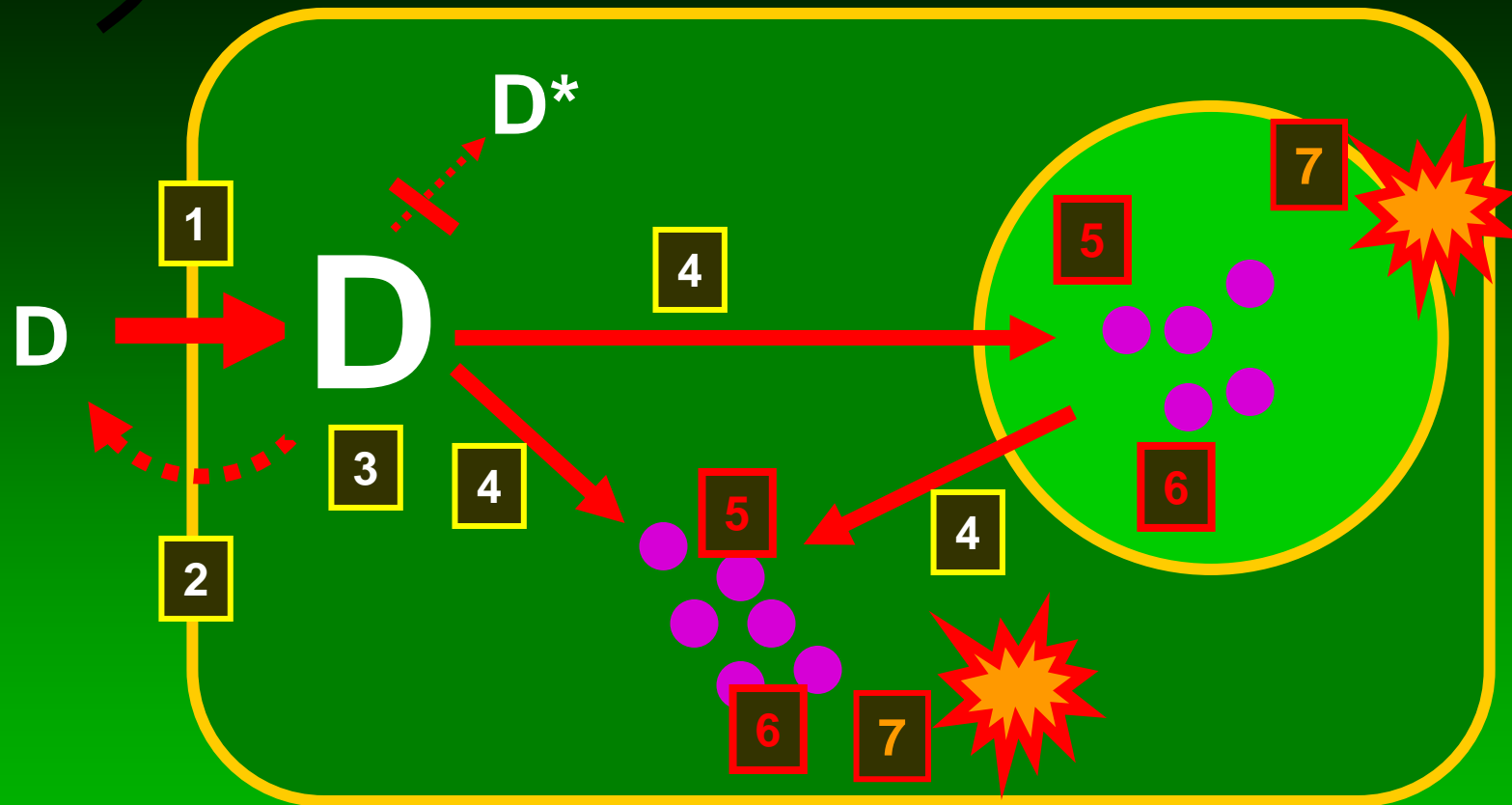
# Any relation to activity ?



1. Penetration
2. No efflux
3. Accumulation
4. Subcell. bioavailability

5. Expression of activity
6. Bacterial responsiveness and pharmacodynamics
7. Cooper. with host def.

# Co-workers on all this stuff ...



M.P. Mingeot, D. Tyteca  
J.M. Michot, C. Seral  
M.B. Carlier, A. Zenebergh  
Y.Chanteux, M. Bouvier d'Yvoire

C. Renard, H. Fan, E. Sonveaux, ...  
S. Carryn, F. Van Bambeke,  
M. Heremans, N. Caceres, ...  
B. Scorneaux, Y. Ouadrhiri,  
I. Paternotte, ....